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NOTES ON REGENERATION.

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During the past summer I made at Woods Holl a number of observations and experiments on the regeneration of several animal forms. The results are here brought together, although they have little more in common than that they all deal with problems of regeneration.

THE LIMITATION OF THE REGENERATIVE POWER OF DENDROCŒLUM LACTEUM.

The fresh-water planarians show such remarkable powers of regeneration that it is surprising to find in one of them, *Dendrocœlum lacteum*, that this power is much reduced. The question at once arises whether we can discover anything peculiar in the relation of this planarian to its surroundings, or in its internal structure that will give a clue to its exceptional behavior.

There is nothing in its habitat to suggest that it has lost, or has never acquired to the same degree, the power of regeneration possessed by other planarians. In the pond at Falmouth where I collected this species there were also present, sticking to the under surfaces of the same stones, both *Planaria maculata* and *Phagocata gracilis*. If *Dendrocœlum* is not as subject to injury as are the other two species, and if, therefore, it does not need the same regenerative power, it is remarkable that *Dendrocœlum* should be so uncommon in comparison with the other two forms. If it is subject to greater injury, then it has not acquired the power to meet the situation as have the other species. Considerations of this kind do not have, I believe, any real bearing on the question of whether an organism has or has not acquired the power to regenerate, although some biologists lay great stress on this sort of speculation. The limitations in the power of regeneration of *Dendrocœlum* are peculiar. Lillie found that when only

the anterior end of the worm is cut off a new anterior end is regenerated. This power to produce a new head was found to extend back to about one-third of the length of the worm, *i. e.*, to a region just in front of the pharynx. Behind this level the posterior piece fails to regenerate a head at its anterior end.

On the other hand, the anterior pieces regenerate a new posterior end from any level, with the possible exception of the immediate region of the head itself; but the latter point has not yet been sufficiently examined in this species. It appears a remarkable fact that this planarian should have such extensive powers of regenerating posteriorly, and such limited powers of regenerating anteriorly, especially since, as far as we know, the same cells produce either a head or a tail according to which end is exposed; but this has not been definitely determined, and would be almost impossible to determine with absolute certainty. Eugen Schultz has also studied the regeneration of *Dendrocœlum lacteum* of Europe[1] and finds that posterior pieces do sometimes regenerate a head, although the regeneration is very slow, and it may appear that Lillie did not keep his pieces a sufficiently long time for the regeneration to take place. He states, in fact, that most of the posterior pieces died after five or six days. Schultz believes that these posterior pieces have potentially the power to regenerate, but that sometimes the piece closes in such a way that the formation of new tissue is prevented, as I have found to occur occasionally in *Bipalium*. Lillie, on the other hand, tries to account for the lack of power of posterior pieces to form a head by means of the following hypothesis. He suggests that the regeneration from the posterior cut surface at all levels is due, in some unexplained way, to the presence "of the brain and anterior part of the nervous system in the anterior piece." Conversely the absence of these structures in posterior pieces is supposed to account for the lack of regeneration from the anterior cut surface. A simple experiment would have shown the untenability of this point of view. If the head end is cut off just in front of the pharynx so that the brain and the anterior part of the nervous system are removed, and then the tail end of the middle

[1] It has been assumed that the European *Dendrocœlum lacteum* and the American form or forms are identical, but I think this question will bear further examination.

piece is also removed, it will be found that the middle piece without regenerating a new head will still regenerate a new tail. This shows conclusively that Lillie's supposition in regard to posterior regeneration is erroneous. The remainder of his argument, which rests on this assumption, also falls, I believe, in the light of this fact.

The great mortality that Lillie observed in the posterior pieces is due largely, at least in my experiments in which the same thing was observed, to the temperature being too high, or possibly to exposure to light. If the pieces are kept cooler (by surrounding the dishes by the cool, running salt water of the laboratory) the mortality is much reduced, and instead of dying after six days, as in Lillie's experiment, I have kept short posterior pieces for several weeks. It is only by keeping such pieces for a long time that one can fairly test their powers of regeneration.

Schultz states that he cut *Dendrocœlum* in two either between the pharynx and the reproductive region or else in front of the pharynx. In the former case he found that the posterior pieces regenerated an anterior end very slowly, and he found it more profitable in studying the regeneration of the head to use those posterior pieces that had been cut off in front of the pharynx. He found that the regeneration of the anterior end often failed to take place, and he attributes this to fusion of the sides of the cut surfaces, as I had found to occur not infrequently in *Bipalium*. Whether this is the whole of the question remains to be seen. In a marine polyclad, *Leptoplana*, Schultz found that posterior pieces, no matter at what level they have been removed, fail to regenerate an anterior end, even when only a small piece of the head is cut off. Yet regeneration from a posterior cut surface takes place at all levels. Schultz attributes the lack of regeneration at the anterior end either to the closing over of the "growing point" by the coming together of the old tissue from the sides, or to the muscles from the sides uniting and thus preventing further growth. Both factors he thinks may enter into the result. This point could be tested, I think, by making the cuts so that there is left a pointed anterior end, when regeneration should occur, if Schultz's view is correct. From an experiment of this sort that I have carried out on *Dendrocœlum* I think it

probable that in *Leptoplana* also no better regeneration would occur, even at a pointed end,[1] and if this proves to be the case Schultz's explanation is insufficient.[2]

In my experiments I first examined whether the form of the cut surface at the anterior end had anything to do with the lack of regeneration, for it was possible here, as in the case of *Bipalium*, that the cross-cut surface closed in such a way that subsequent regeneration was prevented. By changing the form of the cut surface this difficulty should be eliminated. Posterior pieces were cut off through the region of the pharynx and also behind the pharynx. The anterior ends of some of these pieces were very oblique; others were pointed in the middle, *i. e.*, they were cut off by two oblique cuts meeting in the middle line. In the latter case especially it is impossible that the muscles from the sides could close the anterior cut surface.[3] These pieces were kept alive for two or three weeks, and although it could be seen that there was a little new tissue at the anterior cut surface, yet no further regeneration occurred after the first ten days or thereabouts, and there is no indication that regeneration would have gone any further if the pieces had been kept alive for a greater length of time.

Sections of these pieces were made. The results will be given below.

In two other series each worm was cut into three pieces. The head pieces extended to the middle of the region in front of the pharynx. These pieces should be capable of regenerating at the

[1] Loeb says that *Thysanozoon* regenerates a new head, but he did not determine whether a new brain is formed. Monti also obtained regeneration in this form *and also in Leptoplana*, except when cut far posteriorly. Lang also records regeneration in marine polyclads.

[2] Schultz states in the opening of his paper that I carried out my experiments without making sections of the planarians, and he intimates that had I done so I would not have reached certain conclusions in regard to the growth of the new part. How Schultz obtained this information it would be interesting to know. Probably he based his generalization on the absence in my earlier papers of reference to histological details with which I was not then especially concerned. As a matter of fact I had made and studied many sections. My students also were at work on the minute anatomy, and one of them published a complete account of the histological changes taking place during regeneration before Schultz's paper appeared.

[3] Whether union of the dorsal and ventral muscles might close these pieces I have not here considered.

posterior end. The middle pieces included the next portion of the worm, and extended to the region of the reproductive pore. These pieces should be capable of regenerating a head at their anterior ends and a tail at the posterior ends. The third pieces were the tail pieces and included the rest of the worm. These pieces should be incapable of regenerating a head at the anterior end. The pieces were preserved at intervals of 1, 2, 3, 4, 5, 6 days, killed, embedded, stained and examined with immersion lenses.

A study of the sections shows that the changes taking place at the anterior end of the tail-pieces appear to be similar in all respects to those that occur at anterior or posterior surfaces at which regeneration of the missing part takes place. There is nothing in the sections to show why the regeneration should continue in the one case and not in the other, and it is difficult to believe from the evidence of the sections that anterior regeneration from the tail-pieces would not in time be accomplished, yet after three weeks there was no sign of further regeneration and I am forced to conclude with Lillie that in the form of *Dendrocœlum* found at Falmouth regeneration does not, ordinarily at least, occur behind the level of the pharynx. Sections through tail-pieces, cut off behind the pharynx and kept for nearly three weeks, show that the formation of new tissue has not gone much beyond that of the first six days, and that a new head has not been produced. Sections of the oblique, and of the pointed tail-pieces give exactly the same results.

Several writers seem inclined to account for the lack of regeneration in certain planarians, and especially from the posterior region of the body, as due to the absence or small size of the nerve cords in these regions. With this view I do not agree. Lillie has used *Dendrocœlum* as a case in point. Sections of this worm show, however, that the cords in the more posterior regions are as well developed, judging from their size, as they are in *Planaria maculata.*

Regeneration in Pycnogonids.

In 1895 Loeb published some observations that he had made on the regeneration of one of the ·Pycnogonida, *Phoxichilidium*

maxillare. He cut the animal in two between the second and third pairs of legs, and found in two cases that after a time a new part suddenly appeared, presumably after a moult. This new part that regenerated at the posterior end of the anterior piece Loeb speaks of as a body, and points out that this is the first case observed in the arthropods in which new body segments have been seen to regenerate. I have repeated this experiment during two summers, for it did not appear to me beyond dispute that the new part that had been observed was necessarily a body, since no satisfactory evidence that it was such is furnished by Loeb's paper. Although sections of the new part were, apparently, made, no posterior opening of the digestive tract was found, no ganglia are described as being present in the new body, nor do new legs appear to have been present at the sides as we should expect if this new part were really a body.

My first experiments were made in 1901 and, although a number of pycnogonids were kept for two months or longer, none of them regenerated at the posterior end. Since I had used large individuals it seemed not improbable that the lack of regeneration might have been connected with the maturity of the individuals. During the past summer I have repeated the experiment on a large scale, both with large and with small individuals; but although many of the pieces were kept for nearly two months no regeneration took place, with the possible exception of two instances that will be described.

In a number of cases the individuals were cut in two between the third and fourth pairs of legs, *i. e.*, nearer the posterior end than Loeb had cut them, for, from analogy with other cases, it seemed more probable that if the body could regenerate at all it would be more likely to do so the nearer the cut was made to the posterior end. Other individuals were cut in two between the second and third pairs of legs. In only one case did regeneration appear to take place, as shown in Fig. 1. Here the bases of the fourth pairs of legs bulge out as though they had been formed anew, and it seems possible that the rudimentary abdomen is also new, although it is also possible that a part at least of this structure had been left unintentionally when the cut was made. Sections show that the digestive tract opens at the

end of the abdomen. There is no trace of further regeneration within the stumps of the legs. At most, the bases of the legs and the abdomen, or part of the latter, have regenerated.

Fig. 1.

The second case is shown in Fig. 2, in which there is only a bulging out of the end of the body. The cut had been made in this case between the second and third pairs of legs. Sections of this individual do not show any indications of the development

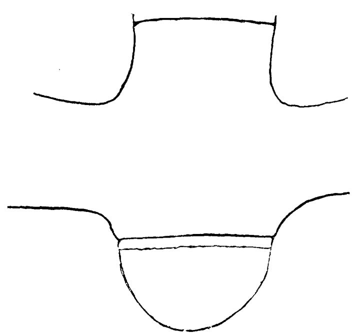

Fig. 2.

of legs or of a rudimentary abdomen in the new tissue of the bulging portion, and there is nothing to indicate that the development would ever have gone any further. The digestive tract ends blindly and is not connected with the ectoderm.

In looking over a large number of individuals I found a few cases in which a leg on one side was much smaller than its

opposite, and from this it seems probable that the original leg had been lost at the breaking joint at the base, and a new one had begun to regenerate. Moreover, I found one case in which the new leg was clearly a new structure, Fig. 3. The different segments had not yet been formed in their adult proportions and

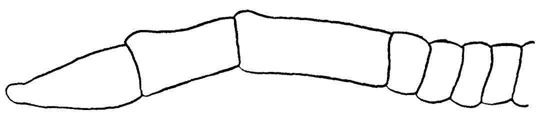

FIG. 3.

the leg could not have been functional as yet. There is some resemblance between this leg and the newly regenerated part from the posterior end of the body that Loeb saw and figured. In fact this idea seems to have suggested itself to Loeb for he writes: "Das Vorhandensein eines ueberzähligen Segmentes könnte vermuthen lassen, dass das neugebildete Stück vielleicht im Laufe der Zeit sich zu einer Extremität entwickelt haben würde, dass es sich also um die Bildung eines Beines an Stelle des abgeschnittenen Rumpfstückes gehandelt habe, ein Fall, den ich als Heteromorphose bezeichnete. Allein Hoek führt an, dass bei Ammotheen das Abdomen nicht selten Spuren einer Segmentation zeigt." Thus in order to explain away the presence of too many segments in the new part Loeb has recourse to a condition found in another species — a mode of explanation that will scarcely recommend itself.

A somewhat fuller analysis of these two cases of Loeb's may not be unprofitable. If the new part is really a body, *i. e.*, thorax and abdomen, we should expect to find the digestive tract opening at the posterior end, but this does not appear to have been the case, for, Loeb says: "Der Darm setzte sich in den vorderen Theil des regenerirten Stückes fort. Im Uebrigen aber waren die Gewebe wenig differenzirt." It is to be remembered that the digestive tract also continues out into the legs in the pycnogonids as a blind sac. In the second place, while the three segments of his first example might be interpreted as representing the two remaining thoracic segments, and the rudimentary abdomen, yet in the other case five or six segments appear in the new part. It is this that led Loeb to suggest that the new part

might represent a leg; but he withdraws this interpretation at once as seen above. There is certainly no striking resemblance between the new part figured by Loeb and the abdomen of *Ammothea*. Finally, if the new part is a new thorax where are the legs?

In the light of these considerations we must wait until some one, favorably situated, has an opportunity to work over the subject with ample materials. Meanwhile it seems to me that so far as the evidence goes it is rather in favor of the view that the regeneration described by Loeb is a new leg and not a part that replaces the lost segments of the thorax and abdomen.

The Lack of Regeneration of the Pigment Spot in the Fin of Fundulus.

If a gold fish having a black band at the end of its tail be selected, and the end of the tail be cut off proximal to the band, a new band like the one removed reappears in the regenerated tail. The presence of black pigment at the cut surface from which the new part regenerates is clearly not necessary for the development of pigment in the new part. This result is all the

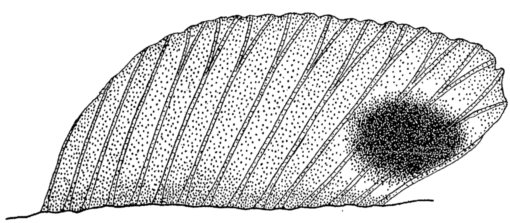

FIG. 4.

more curious since the occurrence of the pigment band is only an individual peculiarity. It seemed desirable to try the same experiment in a species in which a characteristic spot or a ring was present. The dorsal fin of the male of *Fundulus majalis* has a black spot in its posterior part, Fig. 4. The spot is not present in the female, and it appears, therefore, that this color marking belongs to the category of secondary sexual characters.

The posterior part of the fin was cut off by an oblique cut; the part removed containing all of the black spot. The lost part was slowly replaced, and in the course of two months the fin was completed, but the pigment spot did not come back, and there

was no evidence that it would have done so if the fish had been kept longer. Since the operation had been carried out during the height of the breeding season, it seemed possible that the spot might normally fade out later, but other fish, examined in September, showed the spot still present.

The results on *Fundulus* appear to be different from those on the gold fish, and it is not apparent why this difference should exist. The result does not seem to be connected in any way with the fact that the spot in *Fundulus* is a secondary sexual organ. The most plausible explanation that suggests itself is that in the tail of a gold fish that has a black tip there are cells throughout the tail that can develop pigment should they get into the terminal portions of the tail, while no such cells are present in *Fundulus*, or if present they fail to produce pigment in the new part. It may be that in *Fundulus* all the cells capable of producing pigment have been already carried into the pigment-spot itself, and hence when this spot is removed no cells capable of developing this pigment are present in the remaining part. Further work will be necessary to determine whether these suggestions have any value.

The Method of Closure of the Cut Ends of Tubularia.

The peculiar method of closure of the cut ends of *Tubularia* has attracted attention since it appears to be different from the closing observed in other forms. I have already discussed at some length this process [2] and shall not repeat here what has been already said, but since I have observed during the past summer certain processes that seem to throw some light on this question I shall briefly refer to them in this connection. Stevens has figured the closed end of a piece that had been cut through the hydranth-forming region at the time when the primoidium of the new hydranth had just been laid down, and when the red pigment lines, that indicate the appearance of the new hydranth, were present. Over the closed end the red lines radiate to the center of the bounding membrane. It seemed to me that a further examination of pieces that closed in this way might throw some light on the process in general. Pieces were cut off and

[1] Morgan, *Roux's Archiv*, XIV., 1902.

[2] "Regeneration," 1901, p. 69.

kept about twenty-four hours when the primoidia of the new tentacles had begun to appear. At this time I cut the ends squarely off, the plane of section lying across the middle of the new proximal tentacles. To my surprise the cut ends now closed in a very different way from that of ordinary cross-cut pieces. The whole wall contracted from the perisarc and the cut edges were brought together almost at once, and subsequently fused, often showing the radiating lines described by Stevens over the new end. It was perfectly clear that the result was due to a contraction of the cœnosarc, and the difference between this process and that shown by ordinary pieces appears to be due entirely to the fact that at the time when the tentacle primoidia are laid down, the cœnosarc has become free from the outer wall, or perisarc.

From this result it seems to me to follow with great probability that in ordinary pieces the closure of a cut end is also due to a process of contraction of the cœnosarc, but ordinarily the wall of the cœnosarc is so closely stuck to the inner surface of the perisarc that it is not free to pull away as a whole, and there is a consequent drag that holds back the contracting wall, and a consequent modification of the method of closure of the opening. This conclusion also fits in well with some facts observed at the time of closure of the pieces. Certain of the cells that appear to be more closely stuck to the wall are often left behind, or are retarded in their progress towards the center of the newly forming membrane. Thus the peculiar method of closure of *Tubularia* finds its explanation in the unusually close connection between the perisarc and cœnosarc. I have tried to show elsewhere[1] that this same connection may also be responsible for the characteristic "incomplete structures" of *Tubularia*, whose chief peculiarity is that their organs are full sized so far as they are formed.

Transpositional or Compensatory Regeneration of the Large Chelæ in some Crustacea.

Przibram[2] discovered in 1901 in the decapod *Alpheus* that it is possible to cause the small claw (chela) of one side to become

[1] "Some Factors in the Regeneration of Tubularia," *Roux's Archiv*, XIV., 1903.
[2] *Roux's Archiv*, XI., 1901.

the large claw by the simple operation of removing the large claw of the other side. At the next moult the small claw becomes the big one, and the newly regenerated claw becomes the small one. Zeleny[1] found in 1902 that a similar throwing over of the large operculum of the annelid, *Hydroides*, can be brought about by the same sort of operation. Wilson[2] in 1903 made some important additions to Przibram's work, using an American species of *Alpheus*. He suggested that the small claw is merely an arrested stage of development of the big claw, and that when the big claw is removed the check is at the same time taken away that holds back the development of the small claw. At the next moult the small claw becomes the large one, and the new claw the small one.

As yet no one has detected the nature of the correlation that causes the transposition, and this must obviously be the next step in advance. Wilson has suggested that the throwing over is connected with the nervous system, but the experiments on which he bases this suggestion appear to me to be capable also of another interpretation.

During the past summer I undertook some experiments which I hoped would give results bearing on this question, but the outcome has been almost entirely negative. Nevertheless, I shall venture to describe these experiments briefly, because if carried out on more suitable forms they will very probably throw some light on this exceedingly important subject.

Several years ago I found that by cutting the nerve of the leg of the hermit-crab, proximal to the breaking joint, the leg can then be cut off at any level beyond the breaking joint without the remaining part being thrown off at the base. By removing portions of the large leg at different levels, after first cutting the nerve at the base, I hoped to be able to discover whether the amount removed had any effect on the transposition of the large claw to the other side. It was also possible that the simple cutting of the nerve might have some effect, as Wilson's experiment seems to show. The result might also, as Wilson appears to believe, depend in part upon the degree to which the new nerve

[1] *Roux's Archiv*, XIII., 1902.

[2] BIOLOGICAL BULLETIN, IV., 1903.

regenerated before the next moult. In practice, however, it is not possible to cut the nerve without cutting also the blood-vessels, and the injury to the latter may be as important as, or even more so, than that to the nerve.

The experiments were carried out with the hermit-crab and with the fiddler-crab, but were unsuccessful in both cases for different reasons. First, the transposition does not occur under any circumstances in the hermit-crab, as this and other experiments showed; and second, in the fiddler-crabs the muscles, etc., beyond the breaking joint degenerate after the operation. This caused the death of most of the crabs, and those that remained alive had only the outer shell of the leg beyond the breaking joint, and even this fell off in several cases. Since, however, the operation can be carried out in the hermit-crab without the outer part of the leg degenerating, it may be possible, in other forms that have the power of transpositional regeneration (in *Alpheus*, for example), to carry out this experiment successfully.

In both the hermit- and the fiddler-crab I also tried the effect of removing three of the walking legs on the same side of the body as the big claw, leaving the big claw uninjured, in order to see if the absence of the other legs might possibly affect the transposition. This did not succeed, because in the hermit-crabs, as I have said, the big claw does not throw over, and in the fiddlers the experiment had to be brought to an end before any of the crabs had moulted.

All of the individuals of the hermit-crab that I have examined were right-handed, and the shells in which they live have also right-handed spirals. It has been suggested to me that this is an adaptation, in so far that the right-handed hermit crab is placed to better advantage in a right-handed shell. Consequently, if this were true (and I am by no means certain that it is so), it would be disadvantageous for the hermit-crab to have the power of transposition after the loss of the big claw, and in consequence this power has not been acquired, or else, if it existed in the ancestors of the hermit-crabs, it has been lost. That there is really no basis for an argument of this kind is shown by the state of affairs in other decapods; in the lobster, for example. In the American lobster I have seen several cases in which the

big claw had been lost and a new one of the same kind was regenerating on the same side. Przibram has also described cases of this sort. This result is all the more interesting since in the lobster the big claw is present in some individuals on one side, and in other individuals on the other. It cannot be claimed in the lobster that one kind of claw represents an undeveloped stage of the other. In the regeneration of the claws, as especially well seen in the lobster, the particular type of claw is present, although not always fully developed, at an early stage, as Przibram has described, and as I have also found. No doubt the advocates of the view that all beneficial processes have been acquired because of the benefit conferred, will find in these cases of transposition of the big claw from one side to the other evidence of the acquirement of a useful process through natural selection, but I do not think that there is any connection of this sort in these cases.[1]

I have intimated above that the injury to the blood-vessels that run to the leg may be closely connected with the changes that take place in the leg, and account for the absence of transposition in those experiments of Wilson's in which the nerve of the small claw was cut (and presumably also the blood-vessel). My work on the fiddler-crab convinced me that cutting the blood-vessels, which seems nearly always to take place when the nerve is cut, brings about important changes in the condition of the leg. If my suggestion prove correct, namely, that the lack of transposition in Wilson's experiment is due to injury to the blood-vessel rather than to cutting the nerve, then it is possible that the whole phenomenon of transposition may be connected with the condition of the blood supply to the leg. After removal of the large claw more blood may be thrown into the vessel going to the small claw, and this may be the cause of the change that takes place.

[1] In this respect I am in entire agreement with Wilson.